

CLAIMS:

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1. A plasminogen activator amino acid sequence variant that exhibits fibrinolytic activity and contains one or more glycosylation sites at regions that are not glycosylated in the corresponding native plasminogen activator.
2. The variant of claim 1 wherein the glycosylation site is an N-linked glycosylation site.
3. The variant of claim 1 wherein the glycosylation site is an O-linked glycosylation site.
4. The variant of claim 2 wherein the glycosylation site contains a Asn-X-Ser or Asn-X-Thr tripeptidyl sequence of the variant, wherein X is any amino acid except proline.
5. The variant of claim 1 wherein the glycosylation site is within its finger domain, growth factor domain or kringle domain.
6. The variant of claim 4 wherein the glycosylation site is within its finger domain, growth factor domain or kringle domain.

7. The variant of claim 1 wherein the plasminogen activator is human tissue plasminogen activator (t-PA), human urokinase, or human prourokinase.
8. The variant of claim 7 that is t-PA.
9. The variant of claim 4 wherein the plasminogen activator is human tissue plasminogen activator (t-PA), human urokinase, or human prourokinase.
10. The variant of claim 9 that is t-PA.
11. The variant of claim 10 that contains (1) a serine at position 39 of the native t-PA, (2) an asparagine at position 50 of the native t-PA, (3) a serine or threonine at position 60 of the native t-PA, (4) an asparagine at position 64 and a serine or threonine at position 66 of the native t-PA, (5) an asparagine at position 65 and a serine or threonine at position 67 of the native t-PA, (6) an asparagine at position 67 of the native t-PA, (7) an asparagine at position 78 and a serine or threonine at position 80 of the native t-PA, (8) an asparagine at position 79 and a serine or threonine at position 81 of the native t-PA, (9) an asparagine at position 80 and a serine or threonine at position 82 of the native t-PA, or (10) an

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asparagine at position 103 of the native t-PA, or (11) a combination of any two or more of (1) to (10) above.

12. The variant of claim 11 wherein the variant contains an asparagine at position 67 or at position 103 or at both positions of the native t-PA.

13. The variant of claim 5 wherein the plasminogen activator is t-PA.

14. The variant of claim 13 that is modified by functional removal of at least a portion of a domain responsible for fibrin binding but that has restored fibrin binding comparable to that of native t-PA.

15. The variant of claim 13 that is devoid of at least a portion of the finger domain.

16. The variant of claim 15 that comprises natural t-PA devoid of amino acids 1-44.

17. The variant of claim 16 devoid of functional carbohydrate structure at amino acid 184.

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18. The variant of claim 17 still further modified, relative to native t-PA, in the amino acid (1) 205 to 215 region, (2) 244 to 255 region, (3) 233-242 region, or (4) two or more of (1), (2), and (3).

19. The variant of claim 13 that is resistant to specific enzymatic cleavage.

20. The variant of claim 19 essentially free of two-chain form.

21. The variant of claim 20 that is a single-chain mutant.

22. The variant of claim 21 that is stabilized in single-chain form by site-directed mutagenesis at a two-chain cleavage site.

23. The variant of claim 22 wherein position 275 numbered in accordance with the mature plasminogen activator is occupied by an amino acid other than arginine.

24. The variant of claim 23 in which said amino acid is selected from the group consisting of glycine and glutamic acid.

25. The variant of claim 24 in which said amino acid is glutamic acid.

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26. The variant of claim 23 wherein position 277 numbered in accordance with the mature plasminogen activator is occupied by an amino acid other than lysine.

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27. ⁹ The variant of claim ~~26~~ in which said amino acid is isoleucine.

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28. The variant of claim ~~16~~ that is resistant to enzymatic cleavage at the amino acid position 275 or the 277 site or both.

29. The variant of claim 1 capable of exhibiting one or more of the following biological activities: zymogenic activity, fibrin specificity, or plasma clot specificity, characterized in that it contains, in addition to the glycosylation site, an amino acid alteration in its protease domain as compared with the corresponding wild-type t-PA, which alteration is responsible for said biological activity.

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30. The variant of claim 29 wherein the substitution is at position(s) 267, 283+287, 296-299, 303-304, 331-332, 339+342, 347-349+351, 364-366, 408-410, 416-418, 426-427+429-430, 432+434, 440, 445+449, 449+453, 460+462, or 477 of the corresponding wild-type t-PA, where the "+" indicates

alterations only at the positions designated, and the "--" indicates alterations at all positions designated.

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31. The variant of claim 1 selected from the group consisting of
des 1-44N67E275 t-PA, des 1-44N67D184E275 t-PA, des 1-
44N67S184E275 t-PA, des 1-44N67K213E275 t-PA, des 1-
44N67R210A211R212R213E275 t-PA, des 1-44N67R252E275 t-PA, des
1-44N67K210E275 t-PA, des 1-44N67E275I277 t-PA, des 1-
44N67D184E275I277 t-PA, des 1-44N67S184E275I277 t-PA, des 1-
44N67K213E275I277 t-PA, des 1-
44N67R210A211R212R213E275I277 t-PA, des 1-44N67R252E275I277
t-PA, des 1-44N67K210E275I277 t-PA, N67A267 t-PA, N67A283A287
t-PA, N67A296A297AA298A299 t-PA, N67A303A304 t-PA, N67A331A332
t-PA, N67A339A342 t-PA, N67A347A348A349A351 t-PA,
N67A364A365A366 t-PA, N67A408 t-PA, N67A410 t-PA,
N67A416A417A418 t-PA, N67A426A427A429A430 t-PA, N67A432A434
t-PA, N67A440 t-PA, N67A445A449 t-PA, N67A449A453 t-PA,
N67A460A462 t-PA, N67A477 t-PA, N67N103 t-PA, N60N103 t-PA,
N60N67N103 t-PA, des 1-44N103E275 t-PA, des 1-44N103D184E275
t-PA, des 1-44N103S184E275 t-PA, des 1-44N103K213E275 t-PA,
des 1-44N103R210A211R212R213E275 t-PA, des 1-44N103R252E275
t-PA, des 1-44N103K210E275 t-PA, des 1-44N103E275I277 t-PA,
des 1-44N103D184E275I277 t-PA, des 1-44N103S184E275I277 t-
PA, des 1-44N103K213E275I277 t-PA, des 1-
44N103R210A211R212R213E275I277 t-PA, des 1-44N103R252E275I277

t-PA, des 1-44N103K210E275I277 t-PA, N103A267 t-PA,
N103A283A287 t-PA, N103A296A297 A298A299 t-PA, N103A303A304
t-PA, N103A331A332 t-PA, N103A339A342 t-PA,
N103A347A348A349A351 t-PA, N103A364A365A366 t-PA, N103A408 t-
PA, N103A410 t-PA, N103A416A417A418 t-PA, N103A426A427A429A430
t-PA, N103A432A434 t-PA, N103A440 t-PA, N103A445A449 t-PA,
N103A449A453 t-PA, N103A460A462 t-PA, and N103A477 t-PA.

32. A DNA sequence encoding the variant of claim 1.

33. A DNA sequence encoding the variant of claim 2.

34. A DNA sequence encoding the variant of claim 3.

35. A DNA sequence encoding the variant of claim 4.

36. A DNA sequence encoding the variant of claim 5.

37. A DNA sequence encoding the variant of claim 7.

38. A DNA sequence encoding the variant of claim 8.

39. A DNA sequence encoding the variant of claim 11.

40. A DNA sequence encoding the variant of claim 12.
41. A DNA sequence encoding the variant of claim 13.
42. A DNA sequence encoding the variant of claim 16.
43. A DNA sequence encoding the variant of claim 17.
44. A DNA sequence encoding the variant of claim 18.
45. A DNA sequence encoding the variant of claim 23.
46. A DNA sequence encoding the variant of claim 24.
47. A DNA sequence encoding the variant of claim 25.
48. A DNA sequence encoding the variant of claim 26.
49. A DNA sequence encoding the variant of claim 28.
50. A DNA sequence encoding the variant of claim 29.
51. A DNA sequence encoding the variant of claim 30.
52. A DNA sequence encoding the variant of claim 31.

53. A replicable expression vector capable, in a eukaryotic transformant host cell, of expressing the DNA sequence of claim 32.

54. Eukaryotic host cells ~~transformed~~ with the vector of claim 53.

55. The cells of claim 54 that are yeast cells.

56. The cells of claim 54 that are mammalian cells.

57. The cells of claim 56 that are from a Chinese hamster ovary cell line.

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58. A composition for treating a vascular disease or condition comprising a therapeutically effective amount of the plasminogen activator variant of claim 1 in admixture with a pharmaceutically acceptable carrier.

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59. A method of treating a vascular disease or condition in a patient comprising administering the composition of claim 58 to the patient.

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